Bark and Ambrosia Beetles and Their Associated Fungi Colonizing Stressed Walnut in Missouri and Indiana

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Thousand cankers disease (TCD) is a growing threat to black walnut, an economically and ecologically important tree in the eastern US. Trees in the earliest stages of TCD do not display symptoms. In later stages, leaves of the canopy become yellow, wilt, and ultimately die, yet remain attached to their branches. Eventually, all branches in the canopy may die, leading to tree death.

TCD is caused by the fungus, Geosmithia morbida that is carried by walnut twig beetles (*Pityophthorus juglandis*) attracted to walnut trees. After landing on a tree, the beetles bore into the inner bark to create egg galleries. The fungus spreads from galleries and creates small bark cankers. When trees are attacked by a large number of beetles, the fungal cankers can coalesce and lead to development of the disease (Figure 1).

Figure 1— Walnut twig beetle egg galleries and coalescing cankers on a walnut branch (Photo by Ned Tisserat, Colorado State University, bugwood.org)

Declining walnut trees with TCD symptoms were first described in the 1900's in the southwestern US, within the native range of walnut twig beetle. However, the role of the beetles in tree decline was not known at first and decline was blamed on drought. Then in 2009, Colorado scientists made the connection between walnut twig beetles, the pathogenic fungus, and declining black walnut trees. Since that time, the walnut twig beetle and Geosmithia morbida have been detected outside of their native southwestern range, with known occurrences in nine western states and three eastern states, including Tennessee, Pennsylvania, and Virginia. G. morbida infected trees have also been detected in North Carolina but not the beetle, and walnut twig beetles only have been collected from insect traps located at an Ohio mill.

In addition to walnut twig beetles, there are many other types of bark- and wood-boring beetles that create egg galleries in black walnut trees. Most of these beetles carry fungi on their bodies, some of which can be pathogenic to trees. Researchers at the University of Missouri, Purdue University and the Forest Service have teamed up to investigate which bark and ambrosia beetles attack black walnut in the Midwest and to characterize the types of fungi that they carry with them. Bark beetles create egg galleries in the inner bark and ambrosia beetles create egg galleries in the wood. These beetles are close relatives of the walnut twig beetle.

Our research team aims to improve TCD detection in the Midwest and elucidate the possible roles of other beetles and fungi in the disease. Knowing which bark and ambrosia beetles attack black walnut in the native range will aid surveyors in differentiating between walnut twig beetle and other species. Also, knowing which fungi are carried by beetles will determine if we need to monitor for other beetle species that might carry G. morbida or other tree pathogens.

For this investigation of beetles and associated fungi, our team was faced with the question of how to collect enough beetles to study. We decided to take advantage of the fact that bark and ambrosia beetles are attracted to trees that are stressed and declining in vigor. Consequently, researchers created declining "trap" trees. During 2011, twelve trap-tree locations were selected in Missouri and fifteen study sites in Indiana. At each location in Missouri, four black walnut trees were girdled and treated with glyphosate or picloram to induce decline. At each study site in Indiana, two to four black walnut trees were stressed by girdling. Trap trees were left standing for three months to give beetles time to attack. Afterwards, two 12- inch long stem and four branch pieces were removed from the trees and placed in beetle emergence buckets (Figure 2).

Figure 2— Modified 5-gallon buckets used to collect insects that emerged from black walnut logs (Photo by Sharon Reed, University of Missouri).

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Bark and Ambrosia Beetles

Our emergence bucket traps consisted of a five-gallon plastic bucket with most of the bottom cut out with an attached funnel and a transparent collection jar. Logs were end coated with wax and suspended from the lid of the bucket. After adult beetles emerged from logs, they were attracted to light emanating from below the bucket and fell into the collection container. Our team reduced the risk of beetles and the fungi on their bodies desiccating by placing a moistened paper towels in each collection cup.

More than 16,000 bark and ambrosia beetles were collected as they emerged from the logs. Each beetle was identified based on its body shape and its patterns of spines, ridges, and punctures. On the basis of these traits, beetles collected from Missouri and Indiana were sorted into 19 species, with 18 of those species collected in Indiana and nine collected in Missouri. Several species of bark-inhabiting weevils were also collected in our study, but are tentatively considered to be minor or inconsequential to walnut health.

No walnut twig beetles were captured at any of the trap-tree locations. However, a close relative, *Pityophthorus lautus*, was collected at a single trap tree location in Indiana. Although not collected in Missouri during this survey, *P. lautus* beetles have been collected previously throughout Missouri. Fortunately, *P. lautus* beetles are not a black walnut pest and there is no evidence that this beetle can transmit *G. morbida*.

The number of beetles collected varied considerably among the trap tree locations in Missouri and Indiana (Figure 3A and 3B). For example, researchers collected as few as eight beetles from a managed plantation but collected over 2,000 beetles from an abandoned plantation located in the middle of a forest. One reason for some trap-tree locations having more beetles than other sites could be differing amounts of breeding material (nearby dead trees, wood debris) available to beetles for egg laying.

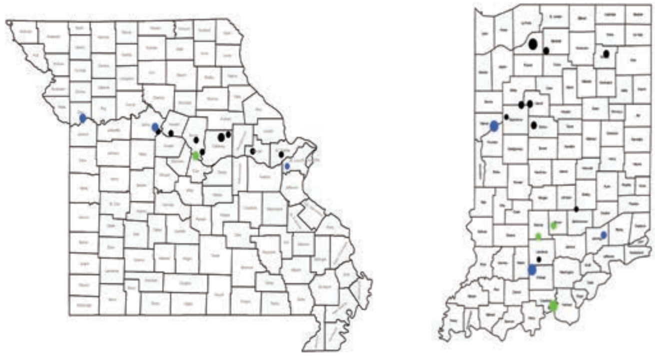

Figure 3A and 3B—Trapping sites in Missouri and Indiana. Small, medium, and large dots represent 1 to 4; 5 to 8; and 9 or more beetle species, respectively, trapped at each location. Black, blue and green dots represent 1 – 500, 501 – 1000, and 1001 – 2500 beetles, respectively. Trapping locations with the most species and beetles captured appear as large, green dots while locations with few species and beetles appear as small, black dots. Figure created by Bill Dijak, US Forest Service.

Most beetles collected in Missouri and Indiana were species of ambrosia beetles that have been introduced to the United States, and therefore are considered “exotic”. Very commonly, exotic beetles arrive on infested wood or wood products imported from countries worldwide. The small size of these beetles makes them very difficult to detect on imported products during port inspections. The majority of exotic beetle species arrive in solid wood packing materials from U.S. trading partners in other countries.

The three most abundant exotic species that were collected from black walnut included the granulate ambrosia beetle (*Xylosandrus crassiusculus*), the black stem borer (*Xylosandrus germanus*), and the fruit-tree pinhole borer (*Xyleborinus saxesenii*). These three beetles are known agricultural pests. In addition, the granulate ambrosia beetle and the black stem borer have been reported to attack and kill stressed black walnut trees, regardless of age. In some cases, young black walnuts attacked by black stem borers will resprout. Young trees attacked by these species may not appear to be stressed. Tree death and dieback are thought to be the result of pathogenic fungi introduced to woody tissues when beetles create egg galleries.

Our team knew that exotic and native ambrosia beetles carry many types of fungi on their bodies but no one had described the full diversity of fungi associated with these beetles. The first step in sorting this out was to isolate fungi from beetles collected from trap-tree logs. A simple procedure was used to isolate fungi from individual beetles selected from each of the four most abundant species. Each selected beetle was ground into small pieces that were spread onto a nutrient agar growth medium that supports growth of *G. morbida* and many other kinds of fungi. Each type of fungus found growing on medium was recorded and purified. Rather than identifying each fungus visually using taxonomic keys, the organisms were identified by molecular methods, using the DNA code unique to each species.

Geosmithia morbida was not among the fungi isolated from 257 beetles; however, a close relative, *G. pallida*, was recovered from two types of ambrosia beetles, the granulate ambrosia beetle and the fruit-tree pin hole borer. The association of *Geosmithia* fungi with ambrosia beetles is not unusual and has been described previously in Europe, Central America, and the US. Although *G. morbida* was not detected, other pathogens were commonly associated with sampled beetles. *Fusarium solani* and multiple Phaeoacremonium species were isolated frequently from beetles. These pathogens are known for their ability to attack many species of woody plants, including nut- bearing trees that have been weakened by drought, flood, and freeze damage.

Other than *Geosmithia pallida*, more than 80 types of fungi were recovered from the bodies of bark and ambrosia beetles. These fungi normally occupy many different habitats such as dead and living plant material, soil, insect guts, and parasitized insects and fungi. Most types of fungi occurred on just a few beetles; however, some fungal types were collected from nearly half or more of beetles belonging to a particular species. These fungal types included Aspergillus, Fusarium, Penicillium,and Phaeoacremonium species. It is uncertain why some types of fungi are associated with beetles more often than others.

The number of fungi found in this survey is likely a conservative estimate of the number of fungi carried by beetles. One reason for this is that not all fungi are capable of growing on the nutrient medium used in the study. In addition, some fungi go undetected because they grow slowly or they are suppressed by combative chemicals produced by other fungi. A different molecular method, also using DNA, is being used by our research team to detect fungi that otherwise would not be detected.

Our survey demonstrated that stressed black walnut trees are an attractive habitat for many types of beetles. These beetles are similar in size and appearance to the walnut twig beetle and could be easily confused by collectors with limited taxonomic training (Figure 4).

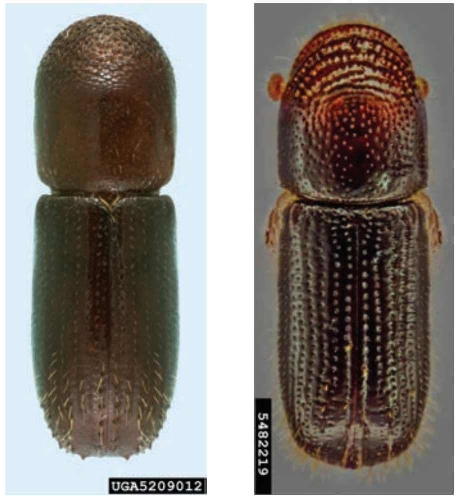

Figure 4—Top view of Xyleborinus saxesenii (left) and Pityophthorus juglandis (right). Photos by Natasha Wright, Florida Dept. of Ag. and Consumer services and Steven Valley, Oregon Dept. of Ag, Bugwood.org, respectively.

In addition, our research team confirmed that all the beetle and weevil species investigated carry a large assortment of fungi on their bodies, including fungi known to be pathogenic to walnut and closely related tree species. The research team is investigating the potential impact of the isolated fungi on black walnut seedlings, especially their ability to cause disease. Further studies are characterizing lesions, cankers and spots found on girdled branches of otherwise healthy black walnut.

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